

# **EXHIBIT 63**

## Possible Role of Ovarian Epithelial Inflammation in Ovarian Cancer

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Ovarian cancer is a commonly fatal disease for which prevention strategies have been limited, in part because of a lack of understanding of the underlying biology. This paper reviews the epidemiologic literature in the English language on risk factors and protective factors for ovarian cancer and proposes a novel hypothesis that a common mechanism underlying this disease is inflammation. Previous hypotheses about the causes of ovarian cancer have attributed risk to an excess number of lifetime ovulations or to elevations in steroid hormones. Inflammation may underlie ovulatory events because an inflammatory reaction is induced during the process of ovulation. Additional risk factors for ovarian cancer, including asbestos and talc exposure, endometriosis (i.e., ectopic implantation of uterine lining tissue), and pelvic inflammatory disease, cannot be directly linked to ovulation or to hormones but do cause local pelvic inflammation. On the other hand, tubal ligation and hysterectomy act as protective factors, perhaps by diminishing the likelihood that the ovarian epithelium will be exposed to environmental initiators of inflammation. Inflammation entails cell damage, oxidative stress, and elevations of cytokines and prostaglandins, all of which may be mutagenic. The possibility that inflammation is a pathophysiologic contributor to the development of ovarian cancer suggests a directed approach to future research [J Natl Cancer Inst 1999;91:1459–67]

Ovarian cancer is the gynecologic cancer most likely to result in death among women (1), yet the pathophysiology underlying epithelial ovarian cancer is not clearly established. For many years, two dominant hypotheses—the ovulation hypothesis (2–4), which relates ovarian cancer risk to incessant ovulation, and the pituitary gonadotropin hypothesis (5), which implicates elevations in gonadotropin levels acting in concert with estrogen—have sought to explain the genesis of this disease. Epidemiologic and biologic data have not been entirely consistent with either of these hypotheses. At the same time, a growing body of epidemiologic evidence suggests that factors causing epithelial inflammation are involved in ovarian carcinogenesis. Such factors include asbestos and talc exposures, endometriosis, and pelvic inflammatory disease (PID). Conversely, there appear to be protective effects of tubal ligation and hysterectomy, which may reduce the exposure from local genital tract irritants. We first briefly review evidence for and against the ovulation and gonadotropin hypotheses. We then propose that inflammation may work in conjunction with, and in addition to, ovulation and steroid hormones in mediating epithelial ovarian cancer risk (Fig. 1).

In this review, only epithelial ovarian cancers will be discussed because they account for about 90% of all ovarian cancers. We will not discriminate between invasive and noninvasive

tumors, since both have similar risk factors. Also, we acknowledge the potential heterogeneity between mucinous and other epithelial ovarian tumor types (6,7), but histology-specific considerations are beyond the scope of this review.

Studies were identified for this review by searching the English language literature in the MEDLINE® database and by an extensive review of bibliographies from articles found through that search.

### EVIDENCE SUPPORTING THE PITUITARY GONADOTROPIN AND OVULATION HYPOTHESES

The factors that afford the greatest overall risk reduction for ovarian cancer in female populations are parity (number of live births) (6,8–36), oral contraceptive use (6,8–16,24,31,32,35–45), and prolonged breast-feeding (31,46). During pregnancy, very high levels of estrogen and progesterone suppress levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and disallow ovulation; during oral contraceptive use, stable levels of estrogens and progestins inhibit the gonadotropins and their ability to stimulate ovulation; and during breast-feeding, low levels of estrogen and LH suppress ovulation (47). That these reproductive and contraceptive factors are protective suggests a common effect through ovulation or steroid hormones. Oral contraceptive use, parity, and breast-feeding each provide a reduction in risk for two to three decades after their cessation, so that they must trigger biologic events that do not clinically manifest themselves as cancer until many years thereafter (48).

If fertility drugs were found to influence the development of ovarian cancer, this influence would also potentially support both the ovulation and gonadotropin hypotheses, since these drugs both elevate gonadotropin levels and cause superovulation. However, the literature (49,50) is conflicting regarding the association between the use of fertility drugs and ovarian cancer.

### SCRUTINIZING THE PITUITARY GONADOTROPIN HYPOTHESIS

The pituitary gonadotropin hypothesis suggests that critical events in the transformation to ovarian cancer are the entrapment

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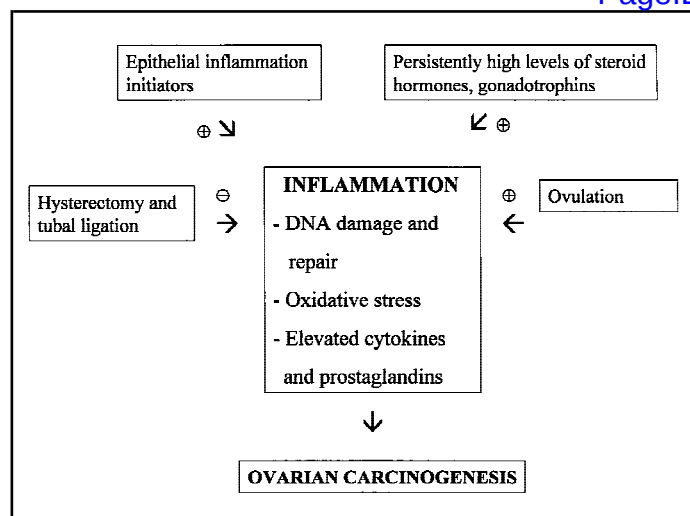


Fig. 1. Inflammation as a common mechanism underlying ovarian cancer.

of surface epithelium in inclusion cysts followed by stimulation of the entrapped epithelium by estrogen or estrogen precursors, particularly in the presence of high and persistent levels of gonadotropins (LH and FSH) (5). Several observations do not completely fit the pituitary gonadotropin hypothesis. High estrogen levels alone could not be the whole story behind mutagenicity because estrogen levels are at their highest during pregnancy, a reproductive event that is strongly protective for ovarian cancer (48). In addition, one study (51) found no estrogen receptors in epithelium on the surface of the ovary or in inclusion cysts. Cramer and Welch (5) illustrated the nature of the proposed interplay between gonadotropins and estrogens and suggested that disruption of negative feedback to the pituitary in the presence of an otherwise normal ovarian steroidal environment (e.g., by transplanting the ovary to the spleen wherein ovarian hormones would be degraded by the liver) would elevate gonadotropins and stimulate ovarian mutagenesis. A pharmacologic equivalent to this would be use of medications, such as barbiturates, halogenated hydrocarbon pesticides, anti-inflammatory medications, and antihistamines, that would degrade estrogen at a greater than normal rate. However, to our knowledge, there has not been any evidence that such medications increase the risk of ovarian cancer (52). These authors (5,53) also proposed that premature ovarian failure or early menopause could be associated with elevated ovarian cancer risk via high gonadotropin levels. However, there is little evidence that age at natural menopause influences risk (32,46).

Furthermore, in the only prospective study to examine this question directly (54), gonadotropin levels measured from serum stored many years prior to outcome were not associated with the occurrence of ovarian cancer. Helzlsouer et al. (54) analyzed levels of LH, FSH, and other hormones among case patients with ovarian cancer and control subjects arising from a prospective population-based serum bank study. Of 20 305 participants from whom serum had been collected and frozen, 31 who were not taking hormone replacement therapy (HRT) at baseline developed ovarian cancer a mean of 8 years after blood collection. These case patients were matched to 62 control subjects on age, menopausal status, and, for premenopausal women, number of days from the beginning of the last menstrual period. Mean levels of FSH, LH, and estrogens were somewhat lower among

case patients with ovarian cancer than among control subjects, whereas the androgens androstenedione, dihydroepiandrosterone, and dihydroepiandrosterone sulfate were associated with an increased risk. These results do not support the hypothesis that elevated pituitary gonadotropin levels increase ovarian cancer risk. However, limitations of the study were the measurement of hormones at a single point in time, the inclusion of premenopausal women without precise determination of timing of blood collection within the menstrual cycle, the small number of cases of ovarian cancer, and the limited adjustment for confounding factors.

A more complex issue that is somewhat difficult to reconcile with the gonadotropin hypothesis is that postmenopausal estrogen use has been modestly, albeit inconsistently, associated with increased risk for ovarian cancer (7,8,11,12,14,18,24,36,55–62). A recent meta-analysis (63), including 11 articles with data from 21 studies, did show a small increase in overall risk with HRT use (relative risk [RR] = 1.15; 95% confidence interval [CI] = 1.05–1.27) with a somewhat higher risk, albeit of borderline significance, among users for more than 10 years' duration (RR = 1.27; 95% CI = 1.00–1.61). Rodriguez et al. (62) in the prospective Nurses Health Study found 18 cases in 5000 person-years among long-term users (>11 years), for an RR of 1.7 (95% CI = 1.1–2.8). Postmenopausal estrogens reduce gonadotropins and increase estrogen levels. To the degree that the gonadotropin hypothesis predicted that excess LH and FSH stimulate mutagenesis, these findings would seem to counter the predictions of the hypothesis. However, if the hormonal mechanism more relevant to the thesis of the gonadotropin hypothesis were that of estrogen elevation, then these findings would indeed fit the data. Taken together, the literature reviewed above does not fully support the gonadotropin hypothesis, although it is quite possible that steroid hormones do play some role in pathogenesis.

## SCRUTINIZING THE OVULATION HYPOTHESIS

The ovulation hypothesis states that excessive ovulation damages the ovarian epithelium, from which epithelial ovarian cancer arises (2). This hypothesis proposes that repeated cell damage translates into an enhanced potential for aberrant DNA repair, inactivation of tumor-suppressor genes, and subsequent mutagenesis (3,4). Perhaps the most complex issue to reconcile with the ovulation hypothesis is whether ovulatory infertility increases the risk for ovarian cancer. Ovulatory infertility is the result of a lack of ovulation and so should not elevate the risk of ovarian cancer according to this hypothesis. Although several studies [reviewed at length elsewhere (49)] have shown that ovarian cancer is associated with difficulty in achieving pregnancy (8,21,31,64–69), there has been inconsistency regarding the type of infertility associated with risk. With regard to ovulatory infertility, Rossing et al. (64) examined records of women who presented to infertility clinics in Seattle, WA, during the period from 1974 through 1985 and who were subsequently identified through cancer registry information if they developed ovarian cancer. Based on small numbers, the RR for ovulatory abnormalities was 3.7 (95% CI = 1.4–8.1) when compared with population-based expected rates of ovarian cancer. This analysis was limited by the likelihood that the external comparison population would likely be more parous, more likely to have used oral contraceptives, and therefore at a lower ovarian cancer risk—hence, resulting in an inflation of the observed RR. In fact, when Rossing et al. compared women with ovulatory infertility with

internal control subjects who had other infertility diagnoses, the risk of ovarian cancer was 1.8 (95% CI = 0.5–6.1). Venn et al. (65) published data from a larger retrospective cohort study of women attending an *in vitro* fertilization clinic and compared their rates with population-based ovarian cancer rates. Again, infertility was associated with ovarian cancer, but only for women with unexplained infertility (odds ratio [OR] = 19.2; 95% CI = 2.2–165) and not for women with ovulatory infertility. In summary, because anovulation is only one among several possible causes of infertility, this limited literature neither supports nor refutes the ovulation hypothesis.

Factors that reduce ovulation do not proportionally reduce the risk of ovarian cancer (24,46). First proposed by Risch et al. (24) and later demonstrated by Whittemore et al. (46), 1 year of delayed menarche or of early menopause was associated with a much less marked reduction in ovarian cancer risk than was 1 year of term pregnancy, 1 year of breast-feeding, or 1 year of oral contraceptive use. Were the ovulation hypothesis to hold, there is no reason to imagine that various sources of ovulation cessation would differentially impact risk. However, age at menarche and age at menopause may less accurately reflect ovulatory function than does pregnancy or oral contraceptive use; the initiation and cessation of menses do not reflect the initiation and cessation of ovulation (70). Nevertheless, suppression of ovulation cannot fully account for the risk reductions observed in epidemiologic studies. Assuming that ovulations occur over a period of at least 20 years, a full-term pregnancy would be expected to reduce ovarian cancer risk by 5%, whereas Whittemore et al. (46) observed about a 15% reduction in risk for each pregnancy after the first.

## EPIDEMIOLOGIC DATA SUPPORTING THE ROLE OF LOCAL INFLAMMATION IN OVARIAN CANCER RISK

Several types of exposure that do not directly affect ovulation or steroid hormone levels but that do enhance local inflammation have been implicated as ovarian cancer risk factors. Reduced passage of inflammatory toxins from the lower to the upper genital tract may also reduce risk.

## TALC AND ASBESTOS EXPOSURE

In the early 1960s, it was recognized that female asbestos workers had an increased risk of developing ovarian cancer and other intra-abdominal neoplasms (71,72). Subsequent retrospective cohort studies of women who were employed in industries wherein they might encounter heavy asbestos exposure (73–75) found about a twofold excess of ovarian cancers over what was expected, with a dose–response relationship suggested. Heller et al. (76) documented that substantial amounts of asbestos fiber could be detected in the ovarian tissues of women whose fathers or husbands worked in occupations in which asbestos exposure was high. The rates of finding asbestos in ovarian tissue were twice as high in women with household exposure as in women without such an exposure history. Animal models (73,77,78) provide some support for the suggestion that asbestos exposure may cause ovarian cancer. Intraperitoneal injection of asbestos into guinea pigs and rabbits results in changes in the ovarian epithelium similar to those seen in early ovarian cancer in women; similar changes were found among 20% of the exposed and 0% of the unexposed animals (77). However, whereas asbestos was cytotoxic to hamster ovary cells *in vitro* (78), it had no effect on the ovaries of mice and hamsters *in vivo* (77).

Although household-related asbestos exposure may be related to dust on the clothing, with those who launder the clothing at increased risk of cancer, it is also possible that exposure occurs through sexual intercourse with particles traveling from the lower to the upper genital tract. Traffic of endogenous cells and pathogens from the lower to the upper genital tract has been shown to be common (79). This fact links cervicitis, i.e., sexually transmitted infection of the lower genital tract epithelium, to PID. It may also link asbestos exposure and talc use to ovarian epithelial inflammation.

Talc, which is structurally similar to asbestos, has repeatedly been related to ovarian cancer. Prior to 1976, talc was commonly contaminated with asbestos, so that the early studies relating talc to ovarian cancer may have been confounded by the asbestos–ovarian cancer relationship (80). More recent findings are less likely to be solely driven by the asbestos relationship.

At least 12 epidemiologic studies (8,81–91) have evaluated the use of talc in relationship to ovarian cancer. Eight of these studies (81–87,90) reported an elevated cancer risk among women whose powder exposure was described as a “dusting of the perineum,” with ORs ranging from 1.3 to 3.9. Two other studies (8,88) found a very small elevation in risk with the use of a more general exposure definition, and one study (89) found no association. In the most extensive and focused analysis to date, Cook et al. (81) interviewed 313 case patients with ovarian cancer and 422 control subjects regarding exposure to a variety of powder products used in a series of ways (e.g., perineal dusting, diaphragm storage, powder on sanitary napkins, and genital deodorant spray). Both talc-containing and non-talc-containing baby or bath powder products were associated with an elevated risk of ovarian cancer; each way of using it, with the exception of diaphragm storage, was also associated with an elevated risk of ovarian cancer. A limited number of studies (8,81,90,92) have examined the potential for a dose–response relationship. Some studies have shown some increase in risk with more frequent exposure (83,86), longer exposure (86), and greater total number of lifetime applications (86). However, other studies (8,81,90) have not shown any dose–response relationship. The link between talc exposure and ovarian cancer is limited by a lack of supportive animal data and an inconsistency in the detection of talc in the ovarian tissue of women who reported heavy use (91). Nevertheless, the consistency of an association between talc use and ovarian cancer in a series of well-conducted studies of varying design suggests that talc use may represent another environmental exposure that enhances epithelial inflammation and thereby either initiates or promotes ovarian carcinogenesis.

## ENDOMETRIOSIS

Endometriosis is the presence of endometrial tissue outside the lining of the uterus. Although the cause of endometriosis is unknown, it is clear that the implantation of ectopic endometrial tissue is associated with a local inflammatory reaction, including macrophage activation, and elevation of cytokines and growth factors.

Ovarian tumors arise out of ovarian endometriosis in 0.3%–0.8% of case patients who are followed clinically (93,94). In the most extensive epidemiologic study to date, Brinton et al. (95) assessed cancer outcomes among 20 686 women with endometriosis who were hospitalized in Sweden. Hospitalizations were identified through the nationwide Swedish Inpatient Registrar, and outcomes were identified through the National Swedish

Cancer Registry after a mean of 11.4 years of follow-up. The risk of ovarian cancer was elevated 2.5-fold for women followed for 10 or more years, and the risk rose to more than fourfold among women whose endometriosis was located in the ovaries. Unfortunately, this study did not control for parity or oral contraceptive use, which might have led to an inflated estimate of risk. However, there is also substantial clinical support for an association between endometriosis and ovarian cancer.

Several case series (93,96–101) have demonstrated cancer tumorigenesis that arises from endometriosis. Sampson (102), who documented the first case, outlined a set of criteria for establishing the existence of such a cancerous transformation. These criteria include the following: 1) demonstration of both cancerous and benign endometrial tissues in the same ovary, 2) demonstration of cancer arising in the tissue and not invading from another source, and 3) demonstration of a histologic relationship between invasive and benign components. Reviewing the literature, Heaps et al. (93) noted that 165 cases have been published that meet these criteria. Almost 80% of these malignant transformations arose from ovarian endometriosis, and the rest came from extragonadal sites. Endometrioid adenocarcinomas accounted for 69% of lesions, followed by clear-cell carcinomas (13.5%) and sarcomas (11.6%). This is a far higher proportion of endometrioid and clear-cell tumors than is found among ovarian cancers in general (10%–20% and 3%–10%, respectively), which again points to a possible transformation from endometriosis to specific types of endometrial cancer (103). One case report (99) documented the experience of a woman who, on biopsy, first showed atypia within ovarian endometriosis and then 3 years later had a clear-cell ovarian carcinoma arising from the same ovary. Finally, Sainz de la Cuesta et al. (96) found endometriosis among about 40% of women with stage I endometrioid or clear-cell ovarian carcinoma, about one third of which were carcinomas arising out of endometriosis. Czernobilsky and Morris (104) also showed that mild cytologic atypia occurred in about 20% of endometriosis lesions and that severe atypia, a probable precursor of ovarian cancer, occurred in 3.6%. Taken as a whole, these data strongly support a temporal pattern of transition from simple endometriosis to atypical endometriosis to ovarian cancer.

## HYSTERECTOMY AND BILATERAL TUBAL LIGATION

Hysterectomy without oophorectomy and tubal ligation both have been associated with reductions in the risk for ovarian cancer (105–115). ORs have ranged from 0.03 to 0.8 for hysterectomy and from 0.2 to 0.9 for tubal ligation. Some authors (105–107) found that the protective effect for hysterectomy waned after 5–20 years and suggested that the observed protection afforded by these procedures might result from screening whereby ovaries examined at the time of surgery and found to be abnormal were removed. However, other authors (6,108,114) found that the protection afforded by hysterectomy or tubal ligation continues for 20–25 years after the procedure. Green et al. (114) proposed that the mechanism whereby hysterectomy and tubal ligation protect against ovarian cancer is by cutting off the pathway between the lower and the upper parts of the genital tract, thereby disallowing proinflammatory exposures from reaching the ovarian epithelium. This may account for the finding by Whittemore et al. (106), who reported no protective effect of hysterectomy in women who had a prior bilateral tubal ligation but found a reduction in risk for women with no prior tubal

ligation. Furthermore, Whittemore et al. showed that tubal ligation protected against the effect of talc. Women who used talc but had never had surgical sterilization were at 30% increased risk of cancer, whereas women who used talc but had a tubal ligation had a 50% reduction in risk. Thus, talc exposure may occur via ascension of particles from the lower to the upper part of the genital tract and tubal ligation severs this route of ovarian exposure. However, the risk reduction associated with tubal ligation or hysterectomy may be larger than would be expected, presuming that these procedures protect the ovarian epithelium from exposure to known inflammants, particularly because only a subset of women is exposed to talc or asbestos. The probable explanation for the fact that risk reduction for tubal ligation hysterectomy is larger than expected lies in the role of as yet unidentified environmental exposures. For example, sexually transmitted pathogens may act via inflammation to increase risk (*see below*). The inflammation hypothesis challenges investigators to search for other exposures that may gain access to the upper genital tract through the lower genital tract and initiate an inflammatory response.

## PELVIC INFLAMMATORY DISEASE

PID is a condition consisting of inflammation of the endometrium, tubes, and ovaries as a result of sexually transmitted infections that ascend from the lower to the upper part of the genital tract. Two case-control studies (34,116) have linked PID with ovarian cancer risk. A third study (117), in which a very small proportion of women (and, therefore, total number of women) reported previous PID, did not. The latter study (117) is likely limited not only by power but also potentially by underreporting of prior PID. Shu et al. (34) first reported a substantial but statistically nonsignificant relationship (OR = 3.0; 95% CI = 0.3–30.2) among a handful of affected case patients and control subjects in Shanghai, China. Risch and Howe (116) subsequently demonstrated the relationship in a study involving 450 case patients with ovarian cancer and 565 control subjects residing in and around Toronto, Canada. They found an increased risk of ovarian cancer among women who had had an episode of PID (OR = 1.5; 95% CI = 1.1–2.1). The relationship between PID and ovarian cancer was most evident in women who had had PID at an early age, were nulliparous, and were infertile. Moreover, there was an increasing trend in risk with increasing number of PID episodes. Each episode of PID promotes a greater and greater inflammatory response, resulting in increasing damage to ovarian and tubal structures and a greater chance of tubal infertility (which, if occurring before the first birth, would manifest itself as nulliparity). Indeed, in the previously mentioned retrospective study of the cohort of infertile women (64), those with tubal infertility were at a threefold increased risk of ovarian cancer. The RR for tubal infertility was of the same order of magnitude as it was for ovulatory infertility, albeit involving a smaller number of individuals and not reaching statistical significance. PID produces infertility by causing inflammation of and damage to the fallopian tube wherein the ovum reaches the uterus, rather than by any effect on ovulation (*see below*). Thus, the finding that PID is associated with ovarian cancer, particularly when there has been resultant chronic inflammation and infertility, is consistent with an inflammatory origin for ovarian cancer.

## ANTI-INFLAMMATORY MEDICATIONS

One way to evaluate the role of inflammation in ovarian cancer is to examine the effect of anti-inflammatory medications on risk. Cramer et al. (52) asked 563 case patients with ovarian cancer and 523 population-based control subjects about their lifetime history of anti-inflammatory medication use. The OR for ovarian cancer associated with at least 6 months of once-per-week aspirin use was 0.75 (95% CI = 0.52–1.10) and for ibuprofen use was 1.03 (95% CI = 0.64–1.64). Limitations of this study included the modest number of case patients exposed to long-term aspirin use and the smaller number exposed to ibuprofen, which resulted in broad CIs around ORs; the inclusion of women with modest use of nonsteroidal anti-inflammatory medications as exposed; and the lack of dose or duration data for aspirin or ibuprofen use. Previous studies showing a protective benefit of aspirin use for colon cancer have typically used a more restrictive definition of exposure, such as aspirin use at least two to three times per week, and have more clearly shown an effect for aspirin use than for other nonsteroidal medications, predominantly because only for aspirin have the number of exposed individuals been sufficient to provide stable estimates (118). Indeed, in the only other published study examining the role of analgesics on ovarian cancer risk (89), among 189 women with epithelial ovarian cancers, the adjusted RR for infrequent use was 0.78 (not statistically significant), whereas the adjusted RR for frequent use was 0.51 ( $P = .05$ ). Thus, further investigation of the impact of anti-inflammatory medications on ovarian cancer is warranted.

## BIOLOGIC RATIONALE FOR THE ROLE OF INFLAMMATION IN OVARIAN CANCER RISK

Ames et al. (119) argued that carcinogenesis in general may be mediated by oxidative damage to DNA. The general theory was based on the finding that mutations in several critical genes, such as the p53 tumor suppressor gene, can lead to tumors. Damage to the DNA constituting these genes may contribute to mutagenicity, to a degree that depends on the degree of damage, the effectiveness of endogenous repair mechanisms, and the rates of cell division. More rapidly dividing cells would be most prone to errors in DNA replication and repair (120).

Inflammation, by its nature, produces toxic oxidants meant to kill pathogens. These oxidants cause direct damage to DNA, proteins, and lipids and may, therefore, play a direct role in carcinogenesis (121). At the same time, chronic inflammation is associated with increased cell division. Rapid cell division gives rise to the potential for replication errors with resultant DNA repair; aberrant DNA repair, particularly at key regulatory sites (e.g., tumor suppressor DNA regions), may increase the risk for mutagenesis (119). Finally, bioactive substances, such as cytokines, growth factors, and prostaglandins, that are synonymous with inflammation may play an important role in ovarian mutagenesis. Ovarian epithelial cells secrete cytokines, including interleukin 1, interleukin 6, and macrophage colony-stimulating factor, among others (122). Auersperg et al. (123) pointed out that these same factors are also produced by ovarian cancer cells and suggested that the recruitment of normally secreted cytokines into dysregulated autocrine loops may be important in neoplastic progression. Prostaglandins have multiple effects that favor tumorigenesis (124). For example, prostaglandins are more common in ovarian malignant tumors than in normal cells (125),

overexpression of prostaglandins increases the invasiveness of tumor cells, and inhibitors of cyclooxygenase activity (and therefore prostaglandin formation) protect against a variety of cancers in animals (124). Epidemiologic studies have shown that long-term use of nonsteroidal anti-inflammatory medications generally reduces the risk of colon cancer in both men and women (118,126,127) and breast cancer in women (128).

Ovulation may be mutagenic. The process of ovulation requires disruption of the ovarian epithelium (129,130). Degenerative epithelial cells adjacent to the site of follicular rupture are shed from the ovarian surface, presumably through apoptosis (i.e., programmed cell death). The wound that ensues from cell loss and follicular extrusion is repaired by the proliferation of epithelial cells from the perimeter of the ruptured follicle. In the process, inclusion cysts are formed as surface epithelial cells become entrapped in the ovarian wound created during ovulation. There has been speculation that inclusion cysts are among the ovarian surface changes that represent a path of differentiation that is less plastic than the relatively pluripotent normal ovarian epithelium and more likely to proceed to ovarian carcinogenesis (130). This suggestion comes from two observations. First, women with ovarian cancer are more likely to have inclusion cysts in the contralateral ovary (131); however, this finding was not confirmed in another study (132). Second, in an unblinded study (133), ovaries of women at high familial risk of developing ovarian cancer, compared with ovaries of normal women, were more likely to have multiple inclusion cysts as well as papillomatosis, deep invaginations, epithelial pseudostratification, and/or hyperactive stroma. Women with a genetic predisposition to ovarian cancer may thus have ovarian epithelium that is already committed to ovarian carcinogenesis, a feature of which is an excess of inclusion cysts.

There are also data from animal studies and limited human studies to support the hypothesis that ovulation may trigger cellular events that result in carcinogenesis. Hyperovulatory hens have a markedly increased likelihood of developing ovarian adenocarcinomas, as do rats with hyperproliferating ovarian epithelial cells (134,135). In women, mutations of the p53 tumor suppressor gene were associated with an increased number of lifetime ovulations in a study by Schildkraut et al. (120). Mutations of the p53 gene are the most common molecular alterations in ovarian cancer and are thought to result from spontaneous errors of DNA synthesis during cell proliferation (136). Risch (137) questioned the validity of these results on the basis that case patients with p53 mutations were older, had poorer tumor differentiation, and had disease of distant rather than of local or regional stage at diagnosis, perhaps indicating that p53-positive tumors are diagnosed later in the neoplastic process. Schildkraut et al. (138) reanalyzed the data matching on age and then on stage and replicated the original findings. However, a more recent case-control study (139) was unable to confirm the association between lifetime ovulations and p53 mutations.

Mutagenicity induced by ovulation may be mediated by inflammation. Ovulation is associated with a marked inflammatory process at the level of ovulatory follicles (140). Many inflammatory mediators, including vasoactive agents such as bradykinin and inflammatory and anti-inflammatory substances such as prostaglandins and leukotrienes, are locally elevated during ovulation. Epithelium in the neighborhood of inclusion cysts is brought in closer proximity to these substances. Follicle rupture probably involves tissue remodeling, with high cell turn-

over, that is also characteristic of inflammatory reactions. Thus, the process of ovulation is intimately related to inflammation. In particular, epithelium in and around the site of ovulation may replicate more actively, come into contact with cytokines and prostaglandins, and may be subject to oxidative stress, thereby enhancing the risk of mutagenesis.

### PREDICTIONS FROM THE INFLAMMATION HYPOTHESIS AND SUGGESTIONS FOR FUTURE RESEARCH

Direct induction of inflammation as a result of endometriosis, talc and asbestos exposure, and PID, as well as ovulation itself, may act to promote ovarian tumorigenesis. There would be several ways to help demonstrate the veracity of this hypothesis. First, anti-inflammatory medications should reduce the occurrence of ovarian cancer. Aspirin use was associated with a reduction in ovarian cancer risk in one previous epidemiologic study; ibuprofen was not (52). Further studies are needed to examine this association. Populations of women with substantial exposures to anti-inflammatory medications, such as those with connective tissue diseases, may be at lower than expected risk, as long as their disease does not inflame the ovarian epithelium. The only study, to our knowledge, that has assessed ovarian cancer risk in a population with connective tissue disease was a relatively retrospective cohort study of patients with rheumatoid arthritis. Cibere et al. (141) examined the observed versus expected rates of numerous cancers among a cohort of 862 Canadian patients with rheumatoid arthritis followed for a mean of 17.4 years. Only five patients developed ovarian cancer, for a standardized incidence ratio of 0.89, which was not statistically significant. Although the number of observed cases was somewhat lower than expected, the number of cases was far too limited for clear interpretation. Larger studies would be of great interest.

Experimentally induced inflammation of the epithelial ovarian surface should be studied to see whether such manipulation will result in epithelial inclusion cysts. Furthermore, demonstration of markers of mutagenicity within inclusion cysts should be sought to suggest movement along a pathway toward ovarian cancer. For example, known markers of mutagenesis, such as mutations in tumor suppressor genes, if they are more common in inflammation-induced inclusion cysts, would provide evidence supporting the role of inflammation in ovarian cancer pathogenesis. Animal experiments could also examine whether suppression of ovarian epithelial inflammation with anti-inflammatory medications would reduce the number of inclusion cysts and the rate of cancer-associated mutations. Antioxidants may also lower ovarian cancer risk, and evaluation of such an effect in both animals and humans would be helpful in testing the inflammation hypothesis.

Susceptibility to the effects of ovarian epithelial inflammation may be modulated by DNA excision and repair potential; i.e., individuals with more precise or active DNA repair capabilities may be relatively spared from the effects of local inflammation. The prevalence of such DNA polymorphisms within women with ovarian cancer and control subjects could be tested. All of these are testable hypotheses that could help in our understanding of the biologic mechanisms underlying ovarian cancer.

It is likely that hypotheses regarding ovulation, gonadotropins, and inflammation are not mutually exclusive but are instead interactive. The occurrence of inflammation during ovu-

lation has been discussed. Steroid hormones may also mediate inflammation. Estrogens, according to the gonadotropin hypothesis, elevate ovarian cancer risk and they may also stimulate the immune response (142). In particular, estrogens have been demonstrated *in vitro* to stimulate B-cell response and decrease suppressor T-cell reactivity, resulting in elevations in antibodies and autoantibodies. Moreover, oral contraceptives elevate the concentrations of local immunoglobulin G and immunoglobulin A in the female genital tract (143). Elevated LH may also enhance oxidative stress. The principal bioassay for LH is the ovarian ascorbic acid depletion assay. Ascorbic acid is an antioxidant, and it is possible that LH depletes ascorbic acid by generating the production of free radicals (144). This observation—that a gonadotropin and estrogen may stimulate inflammation and oxidation—provides a link between steroid hormone excess and the physiologic events involved in inflammation. Thus, it is not necessary to argue as to whether the data fit one hypothesis better than another, but rather it is necessary to develop a more comprehensive model of pathogenesis that may incorporate a role for steroid hormones, ovulation, and inflammation in ovarian cancer. Such a model would account for epidemiologic data suggesting associations between reproductive factors and ovarian cancer and also between PID, endometriosis, talc and asbestos exposure, tubal ligation, and hysterectomy and ovarian cancer.

### SUMMARY

Neither incessant ovulation nor gonadotropin stimulation of ovarian estrogen provides a completely satisfactory explanation for the genesis of ovarian cancer. We have reviewed the data suggesting that an additional mechanism that may underlie ovarian cancer is inflammation, with concomitant rapid DNA turnover and defective repair, oxidative stress, and elevation of bioactive substances. Incessant ovulation, a process that has been linked to ovarian cancer risk, is associated with inflammation at the level of both the epithelium and the follicle. Other factors that cause local pelvic inflammation may also increase risk. Finally, tubal ligation and hysterectomy, which diminish the potential that ovarian epithelium will be exposed to initiators of inflammation, reduce risk. Further observational and experimental data will be needed to confirm the hypothesis that inflammation is a central biologic process in ovarian cancer risk.

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## NOTE

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